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Correspondence

Female-biased gene expression in the malaria mosquito *Anopheles gambiae*

Matthew W. Hahn[1*] and Gregory C. Lanzaro[2]

Females of the malaria-carrying mosquito, *Anopheles gambiae*, must deal with a number of tasks never confronted by males, including blood-feeding and defense against *Plasmodium* infection. Here, we examine global gene expression differences between the sexes in *A. gambiae* via Affymetrix GeneChip® microarrays and find a dramatic over-representation of genes expressed more highly in females. Approximately 10% of the genome has greater than a fourfold difference in expression between males and females, with 71% of these differences being female-biased. In addition to measuring sex bias in gene expression, this is the first experiment to examine expression in the full *A. gambiae* genome. Through comparative genomic analysis with *Drosophila melanogaster*, we find that the functions of sex-biased genes are conserved across the Diptera, even if the same genes do not fill these roles. Unlike *D. melanogaster*, however, there is no evidence for a dearth of male-biased genes on the *A. gambiae* X chromosome.

Hybridization of cRNA from adult, unmated male and female mosquitoes revealed that 4,490 of 14,900 predicted genes had detectable levels of expression in either sex (see Supplemental Data published with this article online). In a comparison of male and female gene expression, we found 2,901 genes that were differentially expressed at $P < 0.05$. Bonferroni correction for the 4,490 tests carried out between males and females results in a set of 167 genes that are differentially expressed at a nominal $P < 0.05$ level (Figure 1). The false discovery rate in this set is extremely low (FDR < 0.00025), and thus we are highly confident that these genes are differentially expressed.

Recent work in *D. melanogaster* has shown that there are many differences in gene expression between the sexes [1–5], and that these dissimilarities are due to differences in both somatic and germline cells [5]. Of the transcripts detected, 17% were differentially expressed at a twofold level between the sexes in *D. melanogaster* [5]; at this level, we found 29% of the genes to differ between males and females (Figure 1). Parisi *et al.* [5] found that there was more male-biased expression among genes in *D. melanogaster*. We found the opposite to be the case in *A. gambiae* — in our high-confidence set of genes, 118 of 167 genes (71%) are expressed more highly in females. This bias appears to get stronger as the difference between the sexes gets larger: in the top 2,000 most-biased genes (by *p*-value), 55% are female-biased; in the top 1,000, 59%; and in the top 500, 63%. The larger difference in sex-biased gene expression in mosquitoes relative to *Drosophila* likely reflects larger differences between the sexes in behavioral (e.g., blood-feeding) and immune (e.g., response to *Plasmodium*) traits in *Anopheles.*

Only 22 of the genes in our high-confidence set were previously annotated. We were able to assign possible functions to 122 of the 167 high-confidence genes by similarity search and found *D. melanogaster* homologs for 96 of them. Four of the *A. gambiae* genes found to be sex-biased in their expression have homologs in *D. melanogaster* that are also sex-biased [5]. Three of the genes are female-biased in both species and one is male-biased in both species (see Supplemental Data).

Though it is hard to draw quantitative conclusions from such a small group of annotated proteins, there are a number of patterns that stand out. A substantial fraction of the female-biased genes (22 of 122) are involved in antigen-related defense, blood-feeding, or are expressed solely in salivary glands [6,7]. Furthermore, many of the biological processes assigned to genes found to be female-biased in *D. melanogaster* [5] are also found in *A. gambiae*: genes encoding proteins involved in ribosomal function, translation initiation, DNA replication and RNA binding, and genes expressed in the ovary. Many of these genes are probably expressed in order to produce eggs in female mosquitoes. While female-biased genes in *A. gambiae* seem to both complement and extend the transcriptionally active set found in female *D. melanogaster*, those genes that are the most male-biased fall into expected categories involved in germline functions [5]. We found that males expressed an abundance of mitochondrial genes, genes encoding protein transport components and heat-shock proteins, and sperm-specific genes, similar to male-biased genes in *D. melanogaster*.

Previous work revealed a significant paucity of genes with male-biased gene expression on the *Drosophila* X chromosome [3], suggesting a role for natural selection in the location of sex-biased genes. Unlike in *D. melanogaster*, however, we found no deficit of male-biased genes on the X chromosome (at any *p*-value cut-off), or indeed any non-random distribution of sex-biased genes on chromosomes; the excess of genes showing high relative expression in females is evenly distributed throughout the mosquito genome (see Supplemental Data).

There also does not appear to be any bias in the movement of genes onto or off of the X chromosome: 53% of all orthologs between *A. gambiae* and *D. melanogaster* remain on the X chromosome after approximately 250 million years [8]. In our dataset three of the five sex-biased genes on the X chromosome in *A. gambiae* that have *D. melanogaster* orthologs

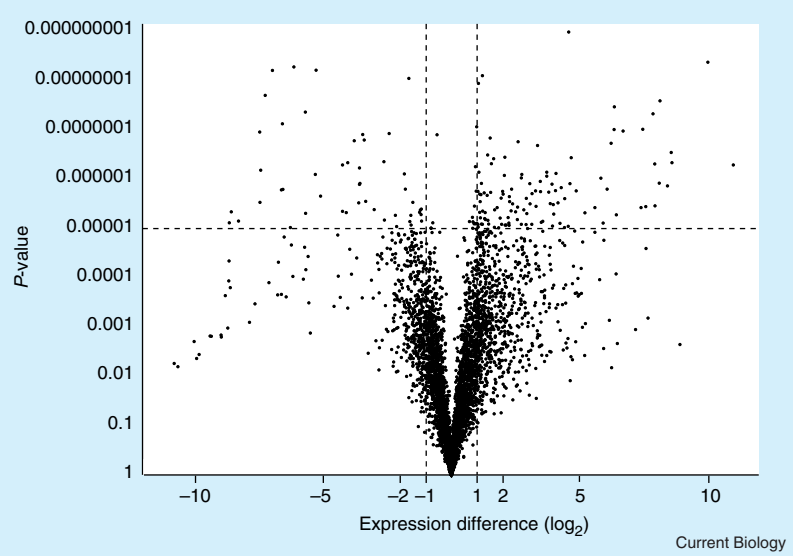

Figure 1. Volcano plots of significance against relative expression differences for the male vs. female comparison.

Each dot represents one of the 4,490 genes that had detectable expression in either sex. The *y* axis is the *P*-value associated with a t-test for differences between the sexes; the horizontal dashed line represents the Bonferroni-corrected threshold for significance, P=0.00001. The *x* axis shows the $\log_2$-transformed difference in expression between male and female mosquitoes; positive values are associated with those genes that are expressed at a higher relative level in females. The vertical dashed lines represent twofold differences in expression.

remain on the X chromosome in *D. melanogaster*. Neither do we find any deficit in apparent sequence conservation of sex-biased genes in *A. gambiae*, as was reported for *D. melanogaster* [3]. Approximately 60% of all mosquito genes have one-to-one orthologs in *D. melanogaster* [8], while we found orthologs for 57% (96/167) of the genes in our high-confidence set (chi-squared = 0.16, P = 0.69).

Although only four of the sex-biased genes in mosquitoes appear to have sex-biased orthologs in *Drosophila*, our comparison shows that the roles played by sex-biased genes — especially in germline function — appear to be conserved. Unlike in *Drosophila* [3], we did not find any evidence for the partitioning of sex-biased genes among chromosomes in *Anopheles*, such as avoidance of the X chromosome by male-biased genes. Various hypotheses have been put forth for both the appeal and the abhorrence of the X chromosome for male-biased gene expression [9,10], but it is unclear why any mechanism should predominate in one or the other species considered here. Examining sex-biased gene expression in another dipteran such as the mosquito *Aedes aegyptii* — where there are no heteromorphic sex chromosomes — may shed light on the fly- or mosquito-specific forces responsible for the observed disparities in sexually dimorphic transcription.

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Supplemental data

Supplemental data including additional discussion, a table of the differentially expressed genes, and experimental procedures are available at http://www.current-biology.com/cgi/content/full/15/6/R192/DC1/

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[1]Center for Population Biology and [2]Department of Entomology, University of California at Davis, California 95616, USA. *E-mail: mwh@indiana.edu